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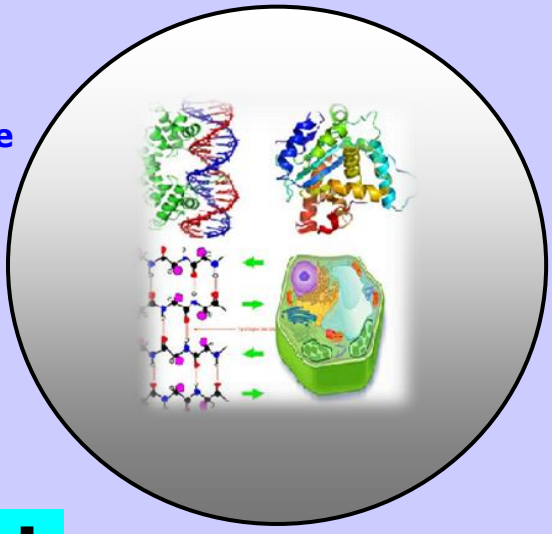

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The Examination of Lactate Acid Bacteria *Streptococcus thermophilus* AST 6 Ability from Fish Digestion Tract to the Different pH

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ABSTRACT

This research was carried out to determine the ability of lactic Acid bacteria (LAB) isolated from fish intestinal tract to pH 1, 2, 3, 4, 5, 6, 7, 8 and 9. Moreover, it is to determine the AST6 LAB growth isolated from fish digestion. Further, this research is beneficial as information for the livestock industries to the use of LAB isolates and is expected to be useful in LAB growth study for stuents, food processing industries, and animal husbandry.
The method used was isolating LAB Streptococcus thermophilus isolates from fish waste in their fish digestion. Then, LAB isolates were incubated in liquid media using MRS broth (5 ml) for 24 hours in difference pH 1, 2, 3, 4, 5, 6, 7, 8 and 9. Positive reaction of growth character LAB isolates was measured the number of optical density (OD) in 620 nm wavelength with spectrophotometer (Spectronic 21; Milton Roy Company, USA).
As stated by Axelson (1998), BAL is mikroaeroleran and asidotoleran. Growth and survival test in certain circumstances is necessary to get the information or character of obtained isolates. The result is BAL AST 6 is able to survive and grow well at low pH.
Keywords: Isolate, Lactic Acid Bacteria, Fish Digestion, pH and Streptococcus thermophilus.

INTRODUCTION

Microflora has important role for digestion tract and the health (Soeyenbos, 1987; Wren, 1987 dalam Soetanto *et al.,* 2001). The balance between beneficial and pathogenic microbial is the important factor in determining product as well and animal health. The type of bacteria *Lactobacilli* and *Streptococci* are microflora which have a role in the balance (Soetanto, 2001). Probiotic term is introduced by Lily dan Stilwell (1965), it is intended as spur growth factor by microorganism. Probiotic comes from Greek which means improving

live (*pro life*). Parker (1974) uses that term for "organism and subtance" which benefits for animal by improving microflora balance. In addition, Fukker (1989) defines probiotic as feed supplement which contains microbial life to improve the balance of digestive tract microbial. Nowadays, probiotic is life microbial consumption as a food additive for health.

(Axelsson, 1998) states that Lactic Acid Bacteria (LAB) are *gram* (+) bacteria, no spore, spherical or rod shaped, negative catalase, *mikroaerotoleran* and *asidotoleran*, produing lactic acid as final product during carbohydrates fermentation. Ecologically, Lactic Acid Bacteria can be isolated from any habitats including human, animal, plant, milk product, meat product, hay, vegetables, and beverages. Since pH has close relation to the enzyme synthesize, it has an effect for the growth, reproduction, and microorganism survival. Most of bacteria optimum acidity (pH) are between 6.5–7.5 but there are species which are capable to grow in extremely sour (asidiphilik) and very alkaline (basophilik). Most species minimum and maximum pH are between 4–9 (Michael J. Pelczar Jr dan E.C.S Chan, 1986). (Fardias, 1989) states that pH affects the activity of enzyme cathalyst for cell biochemical reaction and membrane permeability which protein found in plasma, including enzyme and protein transport.

Generally, lactic acid bacteria are able to grow in pH range 3–5. The pH range is not shown the optimum growth of each LAB type. Knowing the optimum pH for LAB growth associated with optimum activity is needed. The intended activity is lactic acid formation. High level of lactic acid indicates that LAB can be used as probiotic agent.

The requirements of a microorganism can be used as probiotic are:

- Tolerance to the acidity (low pH) and bile.
- Produce lactic acid and anti microbial compounds.
- Able to attach in intestinal wall.
- Resistent to some different environment temperatures especially in digestive tract and processing temperature.

Based on the description above, it can be seen that lactic acid bacteria have pH range (6 – 8) in order to grow optimally. The pH range of each LAB type is difference, so the research dealing with optimum temperature for growth of each LAB isolated from fish digestion is needed.

Formulation of the Problem

How does the growth of LAB isolated from fish digestive tract to some pH range 1, 2, 3, 4, 5, 6, 7, 8, and 9?

The Objectives

To determine the growth of LAB isolated from fish digestive tract to some pH range 1, 2, 3, 4, 5, 6, 7, 8, and 9.

The Significances

1. This research is the basic study which is expected can be used as information source for husbandry industries to the use of LAB isolates.
2. This research is expected to be useful in study relates to the LAB growth for students, food processing industries, and animal husbandry.

MATERIAL AND METHODS

Mikrobial sources

LAB isolated from fish intestinal tract waste.

Chemical materials

Chemical materials used are MRS broth (Oxoid and Pronadisa brand), gelatin, glycerol 20%, lactose, glucose, lactic acid 90%, aquadesh, NaOH dan HCl to adjust the pH, alcohol 70%, generating kit CO_2, methylated spirits, determination lactic acid level reagent, *gram stanning* reagent, H_2O_2, CO_2, and bile salt (Oxoid brand).

A. Tools

1. Tools

(a) Petridish	j) Erlenmeyer	s) Thermometer
(b) Beaker glass	k) Microscope	t) Paper labels
(c) Measuring cup	l) Object glass	u) Cotton
(d) Measuring pipette	m) Ose needle	v) Rubber
(e) Test tube	n) Stove	w) Oven drive
(f) Autoclave	o) Micropipette	x) Spectrometer
(g) Test tube rack	p) Bunsen	y) Cuvet tube
(h) LAF (Laminar Air Flow)	q) Loupe	z) Centrifudge tube
(i) Analytical scales	r) pH meter	

B. Research method

MRS MEDIUM

MRS broth (Oxoid) composition

(a) 1% peptone	e. 0,1% tween 80	i. 0,02% $MgSO_4.7H_2O$
(b) 0,8% lab lemco powder	f. 0,2% dipotasium hydrogen phospate	j. 0,005% $MnSO_4.4H_2O$
(c) 0,4% yeast extract	g. 0,5% sodium asetat. $3H_2O$	
(d) Subtrate (glucose with concentrate based on treatments 0.5%, 1%, 1.5%, 2%)	h. 0,2% citric triamonium	

MRS broth (Pronadisa) composition

(a) 1% bacteriological peptone	e. 0,2% dipotasium hydrogen phosphate	i. 0,02% Magnesium sulfate
(b) 0,8% meat extract	f. 0,1% tween 80	j. 0,005% Mangan
(c) 0,4% yeast extract	g. 0,5% sodium asetat.$3H_2O$	
(d) 2% dextrin	h. 0,2% citric ammonium	

MATERIALS

Lactic Acid Bacteria isolated (LAB**,** MRS broth, TCA 10%, $CuSO_4$ 20 %, distilled water, $Ca(OH)_2$, ice water, para hidroksibipenil solution, concentrated H_2SO_4, alcohol 70%)

Research Variable:

Independent Variables : pH 1, 2, 3 , 4, 5 ,6 , 7, 8, 9.

Dependent Variables : The bacteria growth which measured OD (Optical Density)

Procedures

a. Preparation

- The tools used were sterilized by autoclaving at temperature 121ºC with a pressure 1 atm for 15 minutes.
- MRS broth media: ➔

MRS Liquid Medium:

1. Dissolve 5, 2 gram MRS into 100 ml aquadesh, diluted with strirer until homogoneous.
2. Ensure medium pH 6, 5.
3. Heat medium until boiling in 3 times then divide 4 ml for each tube.
4. Digasi with CO_2 till anaerobic condition then closed and sterilized by by autoclaving at temperature 121ºC with a pressure 1 atm for 15 minutes.

b. Activate LAB isolated from fish digestive tract. Inoculating the culture result as much as 10% in liquid MRS media (MRS broth) 2 x 10 ml. Then, incubate that culture at temperature 37°C for 24 hours.

Lactic acid bacteria isolated from fish digestive tract grown in MRS medium with pH variations.

Alkaline and acidity of MRS medium were obtained by adding HCl and NaOH. HCl addition serves the medium MRS to be acid while NaOH addition serves the medium to be alkaline. pH used in this research are 1 , 2, 3, 4, 5, 6, 7, 8, and 9.

1. Data analysis

Collected data were analyzed descriptively and using One Way *Completely Randomized Design (CRD)*. If there were differences between the average, it would be continued using *Duncan's Multiple Range Test* (DMRT) (Gomez dan Gomez, 1984).

RESULTS AND DISCUSSION

Isolates AST 6 survival test at pH 1, 2, 3, 4, 5, 6, 7, 8 and 9. As seen in the Table 1.

From the growth and survival test in certain circumstances, the character of obtained isolates (LAB AST 6) is able to survive and grow well at low pH. As stated by Axelson (1998), BAL is mikroaeroleran and asidotoleran.

pH affects the lactic acid bacterias' activity. Lactic acid production will increase in line with the increasing of pH until certain limit (Paat, 1999). Lactic acid bacteria produce lactic acid as primary metabolite in logarithmic growth phase (tropophase) having important role in essential macromolecule and enzyme arranging. Rapid cell growth and primary metabolite production in tropophase will accelerate to the stationary phase (Borris, 1988).

Acid production is followed by an increase (H^+) or decrease pH, so the tolerance to the acid correlation becomes easy for the lactic acid bacteria survival. Not all the bacteria require acid condition to initiate growth reaction. Generally, rod-shaped LAB are more acid acceptably than round-shaped (Axelsson, 1998). (Daly et al, 1992) state the role of organic and anorganic compounds in bacterias' growth. Organic compounds play role in growth pattern directing. Anorganic compound H_2O_2 inhibits microbial while CO_2 simultaneously emits O_2 (dual effect) which produce quick fermentation and protect labile component of the O_2 (ascorbic acid).

Tabel. 1. Survival LAB AST6 to pH

	Jam Ke-									
	0	0,5	1	2	3	4	5	6	7	8
pH 1	0,273	0,294	0,294	0,300	0,311	0,295	0,302	0,302	0,303	0,302
pH 2	0,310	0,330	0,317	0,349	0,358	0,361	0,370	0,357	0,363	0,385
pH 3	0,274	0,265	0,261	0,264	0,264	0,260	0,264	0,256	0,255	0,251
pH 4	0,227	0,224	0,223	0,220	0,221	0,217	0,217	0,219	0,219	0,210
pH 5	0,199	0,201	0,206	0,247	0,240	0,267	0,298	0,340	0,366	0,410
pH 6	0,204	0,219	0,237	0,362	0,426	0,592	0,702	0,837	0,903	0,934
pH 7	0,218	0,271	0,302	0,495	0,688	0,926	1,035	1,120	1,154	1,171
pH 8	0,222	0,269	0,315	0,517	0,857	1,092	1,210	1,273	1,294	1,297
pH 9	0,196	0,242	0,309	0,616	1,014	1,302	1,402	1,443	1,463	1,472

	Jam Ke-									
	10	12	14	16	18	19	21	22	23	24
pH 1	0,310	0,302	0,301	0,298	0,290	0,290	0,290	0,291	0,290	0,292
pH 2	0,382	0,404	0,374	0,336	0,346	0,379	0,389	0,382	0,384	0,385
pH 3	0,254	0,257	0,244	0,246	0,238	0,242	0,244	0,245	0,245	0,243
pH 4	0,220	0,219	0,215	0,214	0,139	0,140	0,140	0,139	0,139	0,138
pH 5	0,480	0,536	0,589	0,610	0,624	0,644	0,644	0,692	0,652	0,655
pH 6	1,015	1,088	1,035	1,039	1,038	1,057	1,063	1,058	1,064	1,071
pH 7	1,186	1,206	1,204	1,216	1,224	1,241	1,254	1,256	1,260	1,269
pH 8	1,314	1,334	1,347	1,364	1,373	1,380	1,398	1,402	1,403	1,407
pH 9	1,474	1,493	1,494	1,512	1,519	1,523	1,537	1,541	1,543	1,547

Most bacteria optimum acidity (pH) are between 6.5–7.5 but there are species which are capable to grow in extremely sour (asidiphilik) and very alkaline (basophilik). (Michael J. Pelczar Jr dan E.C.S Chan, 1986) state that the most species minimum and maximum pH are between 4–9, while lactic acid bacteria are capable to grow in pH range 3-5. Lactic acid production requires large amounts of good nutrients in order to enzim biosynthesis for cell growth. Bacteria cell growth needs nutrient, nitrogen, incubation temperature, medium pH, and incubation length (Fardiaz, 1988).

Ecologically, Lactic Acid Bacteria can be isolated from any habitats including human, animal, plant, milk product, meat product, hay, vegetables, and beverages.

Although these microorganism exist in various places but a few species adapts specific environment, such as, is only able to grow on the salt levels, sugar, higher alcohol, pH 3,0 – 8,0, and is able to ferment various types of mono and disaccharide. Some species are capable to form antagonistic compounds to LAB which dominate their environment (Axelsson, 1998).

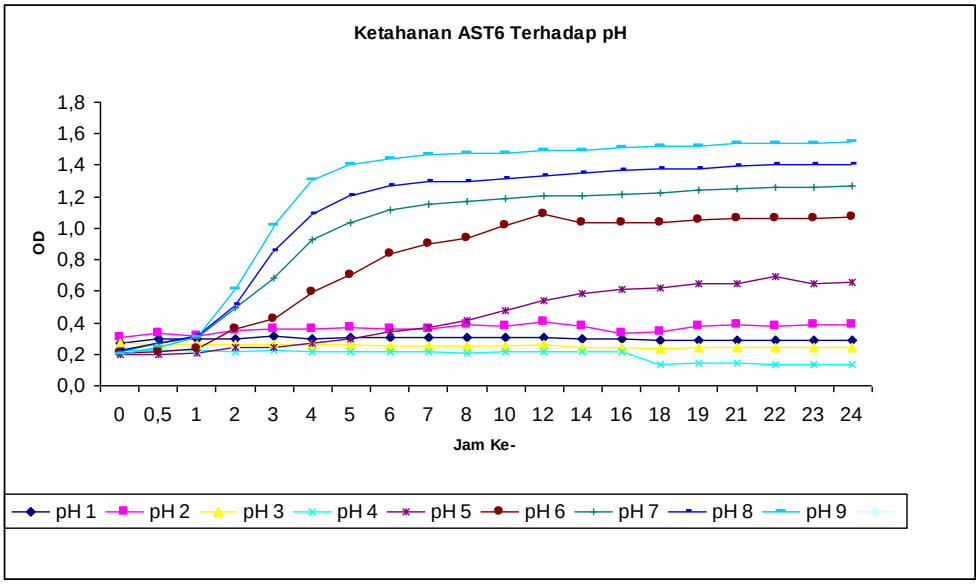

Figure 1. Survival AST6 to pH Graphic.

KESIMPULAN

The results of this research are LAB from fish digestive tract are able to survive at low pH (3) and AST 6 LAB can be probiotic agent.

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